Effects of angiotensin II type-1 receptor blocker losartan on age-related cardiovascular risk in spontaneously hypertensive rats

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Abstract. Ageing and hypertension are the major risk factors for the development of cardiovascular and renal diseases, and the renin-angiotensin system has been shown responsible for these pathologies. Thus, the aim of this study was to compare the effects of losartan, angiotensin II type-1 receptor blocker, on systolic (SBP), diastolic (DBP), and mean (MBP) blood pressure, pulse pressure (PP) and heart rate as well as regional haemodynamics, cardiac hypertrophy and biochemical parameters in adult (L9: 9-month-old) and aged (L18: 18-month-old) spontaneously hypertensive rats (SHRs). Aged match untreated SHRs served as controls (U9: 9-month-old and U18: 18-month-old). Aortal blood flow and resistance were significantly improved by losartan treatment in L9 vs. U9 \((p<0.05)\). In aged SHRs, losartan significantly reduced SBP, MBP, PP, right ventricle weight index, and improved age-related impairment of left ventricular weight index (U18: 4.21 ± 0.09 mg/g vs. U9: 3.54 ± 0.34 mg/g, \(p<0.05\) and vs. L18: 3.65 ± 0.07 mg/g, \(p<0.001\)), carotid, renal, and aortal vascular resistance, and glomerular filtration rate (U18: 2.75 ± 0.27 ml/min/kg vs. U9: 4.84 ± 0.85 ml/min/kg, \(p<0.05\) and vs. L18: 3.65 ± 0.07 ml/min/kg, \(p<0.05\)). These results demonstrate significantly impaired systemic and regional haemodynamics and left ventricular hypertrophy in old SHRs. Losartan decreased age and hypertension associated cardiovascular risk by decreasing vascular resistance and pressure overload, ventricular hypertrophy, and preserving kidney function.

Key words: Hypertension — Ageing — Cardiovascular risk — Losartan

Introduction

Cardiovascular complications, associated with ageing and/or hypertension, are caused by alterations in vascular structure and function (Taddei et al. 1997). High blood pressure (BP) is a powerful cardiovascular (CV) risk factor that acts on the arterial wall and is responsible in part for various CV events, such as cerebrovascular accidents and ischemic heart disease (Safar et al. 2003). Systolic and diastolic dysfunctions are independent CV risk factors in the elderly patients, while pulse pressure (PP) is a powerful predictor of CV events in this population (Van Bortel et al. 2001; Fyhrquist et al. 2005). In essential hypertension haemodynamic alterations characterized by increased resistance in peripheral circulation are a consequence of structural, mechanical and functional changes in peripheral vasculature. Advanced age is associated with many CV pathophysiological states, including arteriosclerosis (Iwamoto et al. 2000), stroke (Arboix et al. 2006), heart failure and coronary artery disease (Susic et al. 1999b), and progressive renal damage (Mihailovic-Stanojevic et al. 2003). Age-associated remodeling of the walls of large arteries of rodents, nonhuman primates and humans includes luminal dilation, intimal and medial thickening, vascular stiffening, and endothelial dysfunction (Lakatta 2003). In addition, increase in intima-media thickness of the common carotid artery that occurred with ageing are associated with a number of CV complications and therefore can be predictive and independent factor of CV risk (Ariff et al. 2002). Left ventricular hypertrophy (LVH), ventricular fibrosis and impaired ventricular performances are cardiac manifestations of both hypertension and ageing, and components of the ventricle are affected in hyperten-
sive heart disease as well as in ageing heart (Varagic et al. 2001a). The spontaneously hypertensive rats (SHRs) have been extensively studied as a model of cardiac hypertrophy that results from genetically-induced pressure overload and leads to the development of failure in advanced age (Brooks et al. 1997).

On the other hand, Komatsu (Komatsu et al. 1995) has demonstrated that the 73-week-old SHR naturally developed all pathophysiological and clinical alterations that are associated with end stage renal disease (ESRD) in patients with essential hypertension. Decrease in total renal blood flow (RBF) along with structural and functional changes of the kidney of this rat strain further induces the progression of renal failure to ESRD (Mihailovic-Stanojevic et al. 2003).

Numerous studies confirmed the important role of the renin-angiotensin system in the domain of cardiovascular and renal physiology and pathophysiology. Angiotensin II (Ang II), the effector peptide of the renin-angiotensin system, exerts a variety of actions in physiological BP and body fluid regulation, and has also been implicated as a pathogenic factor, via angiotensin type-1 (AT1) receptor, in the development of cardiovascular (Pratt 1999; Unger 2001) and renal diseases (Hall et al. 1999). In ageing endothelial cells, production of vasoconstricting growth factors such as Ang II and endothelin-1 (ET-1) are increased, and that of vaso-dilatory factors (e.g. nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factor) are reduced (Najjar et al. 2005). Previously we showed that losartan successfully protect kidney vessels against ageing and hypertension-induced dysfunction and remodeling by reducing hypertrophy and hyperplasia of vascular smooth muscle cells and by attenuating abnormal expressions α-SMA (an indicator of mesangial activation and renal fibrosis) (Mihailovic-Stanojevic et al. 2004).

Previous studies from others in young and adult animals have shown that hypertensive CV changes are reversible, while only a few studies with antihypertensive agents were processed in aged SHR (Susic et al. 1999a), being more or less effective in improving heart, renal, and vascular changes, depending on drags that were used and/or duration of treatment in those studies (Oddie et al. 1993; Brilla et al. 1996; Linz et al. 1999; Cerbai et al. 2000; Linz et al. 2000; Tsoetser et al. 2001).

In this study we compared the effects of Ang II AT1 receptor antagonist, losartan on hypertension/ageing-related changes of systemic (systolic, diastolic, mean, pulse pressure and heart rate) and regional (carotid, aortal, and renal) haemodynamics, cardiac hypertrophy and biochemical parameters in adult and aged SHRs. We have now investigated whether 4-week losartan treatment could reduce hypertension and ageing-induced heart, renal and vascular changes, and thus decrease age-related CV risk in SHR.

### Materials and Methods

#### Animals

Male SHRs, 9- and 18-months-old, obtained in this study were bred at the Institute for Medical Research (Belgrade, Serbia) and were descendants of breeders originally obtained through Taconic Farms (Germantown, NY, USA). They were maintained in temperature and humidity controlled rooms on a 12 : 12 h light/dark cycle. Animals were fed with standard laboratory chow (Veterinary Center Subotica) and water *ad libitum*. All experiments were done according to our Institutional guidelines for animal research and principals of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other (Official Daily N. L. 358/1-358/6, December 18, 1986).

#### Experimental protocol

Animals were divided into four groups: untreated groups (9- and 18-month-old) received tap water, while treated SHR (9- and 18-month-old) received losartan dissolved in tap water (DUP 153, Du Pont, Wilmington, DE, USA; 10 mg/kg b.w./day) by gavage during four weeks of experiment.

Before haemodynamic measurements all animals were weight and placed in metabolic cages during 24 h for collection of urine for biochemical analysis and measurement of urine flow.

All procedures were done under general anesthesia (i.p. sodium-pentobarbital 35 mg/kg b.w.). Left femoral artery was cannulated by polyethylene catheter (P-50, Clay-Adams, Parsippany, NJ, USA) and connected to physiograph by low-volume displacement transducer (P23 Db; Statham, Oxnard, CA, USA) and a direct writing recorder (Physiograph Four; Nacro Bio System, Inc.) for continuous BP measurement. Systolic (SBP) and diastolic (DBP) blood pressure were measured directly, and mean blood pressure (MBP) was obtained by electronic integration, while heart rate was recorded at the same time. PP was displayed as the difference between SBP and DBP. For regional blood flow measurements left carotid artery was gently separated from the surrounding tissue. An ultrasonic flowprobe, 1RB (internal diameter = 1 mm) was placed around the artery and total carotid blood flow (CBF) was recorded using a Transonic T106 small animal flowmeter (Transonic System Inc., Ithaca, NY, USA). After abdominal incision renal artery preparation was utilized and RBF was recorded. Vascular resistance in these two vascular beds (carotid vascular resistance (CVR) and renal vascular resistance (RVR)) were calculated by dividing mean arterial pressure with total blood flow through
respective blood vessel, normalized for the body weight and expressed as mmHg-min-kg/ml. The segment of the abdominal aorta under the bifurcation of the renal artery was carefully prepared and aortal blood flow (ABF) was measured at the same procedure as for CBF and RBF, but with ultrasonic flowprobe, 2RB (internal diameter = 2 mm) and the aortal vascular resistance (AVR) was calculated as described above.

At the end of the study, blood samples were collected for the biochemical analysis. Urinary and plasma total protein and creatinine concentrations, and total plasma cholesterol and triglyceride levels were determined using Cobas Mira, Rosh analyzer and Cobas Integra 400 Plus (Elitech Diagnostic). Standard formula was used to calculate protein excretion, urine protein/creatinine ratio and creatinine clearance as estimation of renal function.

After that, animals were sacrificed and the heart was removed immediately. The atria were dissected free from the ventricles and discarded. The free wall of the right ventricle (RV) was separated carefully from the left ventricle (LV). Wet weight (g) of the both ventricles was measured, and corrected for the body weight and expressed as ventricular weight indexes (mg/g).

Statistical analysis

Results are expressed as mean ± S.E.M. One way analysis of variance (ANOVA) and unpaired Student’s *t*-test was applied as appropriate. The differences between examined groups were considered being significant if *p* < 0.05.

Results

**Haemodynamic parameters**

Chronic losartan treatment significantly reduced SBP, MBP, and PP in the aged SHR. On the other hand, in the adult SHR, given losartan during four weeks resulted in slight, but not significant decrease in SBP, DBP and MBP without effects on heart rate and PP (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>U9 (n = 6)</th>
<th>L9 (n = 7)</th>
<th>U18 (n = 10)</th>
<th>L18 (n = 13)</th>
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<tr>
<td>SBP (mmHg)</td>
<td>194.00 ± 10.91</td>
<td>174.43 ± 6.89</td>
<td>197.80 ± 8.84</td>
<td>147.85 ± 12.45</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>129.17 ± 13.15</td>
<td>110.86 ± 6.45</td>
<td>136.90 ± 8.94</td>
<td>98.46 ± 11.16</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>163.36 ± 17.07</td>
<td>133.32 ± 7.35</td>
<td>157.90 ± 9.14</td>
<td>114.15 ± 12.39</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>64.83 ± 3.89</td>
<td>63.57 ± 5.53</td>
<td>60.90 ± 6.61</td>
<td>49.38 ± 3.76</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>388.33 ± 10.46</td>
<td>364.29 ± 7.82</td>
<td>347.40 ± 12.16</td>
<td>345.92 ± 8.81</td>
</tr>
</tbody>
</table>

U9 and U18, untreated SHRs 9 and 18 months of age; L9 and L18, 9 and 18 months aged SHRs treated with losartan during four weeks of experiment; *p* < 0.05 vs. the age match untreated SHRs; *p* < 0.05 vs. the adult SHRs.

**Figure 1.** Carotid blood flow (CBF) and carotid vascular resistance (CVR) in SHRs. U9 and U18, untreated SHRs 9 and 18 months of age; L9 and L18, 9 and 18 months aged SHRs treated with losartan during four weeks of experiment; *p* < 0.05 vs. the age match untreated SHRs; *p* < 0.05 vs. the adult SHRs.
Age-related cardiovascular risk

Body and LV and RV weight

No difference in body weight was found among the experimental groups (Table 2). Left and right ventricular weight and ventricular weight indexes were significantly (LV: $p < 0.01$; LVI: $p < 0.001$; RV: $p < 0.05$; RVI: $p < 0.05$) lower in losartan-treated aged SHR than in untreated controls (Table 3). There were no differences in LV and RV weight and ventricular weight indexes between adult losartan-treated and untreated SHR (Table 3).

Biochemical parameters

Biochemical parameters are shown in (Table 2). The total plasma cholesterol and triglyceride levels were significantly increased in aged versus adult SHR, and losartan did not affect either of these parameters. Plasma creatinine level was significantly elevated, while urine creatinine concentration was significantly reduced in untreated aged, compared to adult SHR, thus creatinine clearance dropped significantly.

| Table 2. Body weight (b.w.), triglyceride (Trg), cholesterol (Chol), plasma creatinine (PCr) and urine creatinine (UCr) concentrations, total plasma protein (TP), urine flow (Uf), protein excretion (Pexc), creatinine clearance (CCr) and urine protein/creatinine ratio (UP/Cr) in SHRs |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | $U_9$ ($n = 7$) | $L_9$ ($n = 7$) | $U_{18}$ ($n = 10$) | $L_{18}$ ($n = 13$) |
| b.w. (g)        | 302.86 ± 9.93   | 291.43 ± 13.17  | 308.50 ± 10.11   | 307.50 ± 9.26   |
| Trg (mmol/l)    | 0.35 ± 0.04     | 0.38 ± 0.08     | 1.33 ± 0.2       | 1.32 ± 0.27     |
| Chol (mmol/l)   | 0.70 ± 0.04     | 0.65 ± 0.05     | 1.28 ± 0.07      | 1.25 ± 0.09     |
| PCr (μmol/l)    | 48.71 ± 1.34    | 51.57 ± 1.13    | 54.71 ± 1.99     | 54.40 ± 2.73    |
| TP (g/l)        | 66.77 ± 0.99    | 73.47 ± 2.67    | 61.03 ± 4.06     | 55.66 ± 1.66    |
| UCr (mmol/l)    | 10.56 ± 1.82    | 10.59 ± 1.64    | 3.22 ± 0.34      | 4.84 ± 0.55     |
| Uf (μl/min/kg)  | 23.36 ± 2.58    | 20.78 ± 2.47    | 47.07 ± 2.21     | 43.86 ± 2.56    |
| Pexc (mg/min/kg)| 0.18 ± 0.02     | 0.15 ± 0.02     | 0.39 ± 0.05      | 0.41 ± 0.08     |
| CCr (ml/min/kg) | 4.84 ± 0.85     | 4.01 ± 1.25     | 2.75 ± 0.27      | 3.84 ± 0.26     |
| UP/Cr (mg/mg)   | 7.43 ± 0.77     | 6.55 ± 0.53     | 24.22 ± 2.85     | 17.69 ± 2.63    |

$U_9$ and $U_{18}$, untreated SHRs 9 and 18 months of age; $L_9$ and $L_{18}$, 9 and 18 months aged SHRs treated with losartan during four weeks of experiment; * $p < 0.05$ vs. the age match untreated SHRs; ** $p < 0.01$, *** $p < 0.001$ vs. the adult SHRs.
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Table 3. Left and right ventricular (LV and RV) weight and weight indexes (LVI and RVI) in SHRs

<table>
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</tr>
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<tbody>
<tr>
<td>LV (g)</td>
<td>1.06 ± 0.10</td>
<td>1.09 ± 0.08</td>
<td>1.29 ± 0.04</td>
<td>1.14 ± 0.04</td>
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<tr>
<td>RV (g)</td>
<td>0.26 ± 0.06</td>
<td>0.24 ± 0.04</td>
<td>0.27 ± 0.02</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>LVI (mg/g)</td>
<td>3.54 ± 0.34</td>
<td>3.74 ± 0.25</td>
<td>4.21 ± 0.09</td>
<td>3.65 ± 0.07</td>
</tr>
<tr>
<td>RVI (mg/g)</td>
<td>0.87 ± 0.18</td>
<td>0.82 ± 0.15</td>
<td>0.88 ± 0.06</td>
<td>0.71 ± 0.03</td>
</tr>
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(U9 and U18, untreated SHRs 9 and 18 months of age; L9 and L18, 9 and 18 months aged SHRs treated with losartan during four weeks of experiment; * p < 0.05, ** p < 0.01, *** p < 0.001 vs. the age match untreated SHRs; † p < 0.05, vs. the adult SHRs.

Discussion

The data from the present study demonstrated that 4-week AT1 receptor blockade with losartan (10 mg/kg/day) improved BP, LVH carotid and aortic haemodynamics and preserved renal function and therefore decreased age-related CV risk in hypertensive rats. We also showed that Ang II is involved in RV remodeling in old SHR, because losartan treatment significantly reduced RV weight and RVI. Likewise, we demonstrated that in adult SHRs, when age-related cardiac and renal failure have not been completely developed yet, prolonged treatment with losartan resulted in marked but not significant reduction of urine protein/creatinine ratio in aged SHR, but the difference was not significant.

(p < 0.05) in this group versus values in untreated adults. Chronic losartan treatment significantly increased urine creatinine concentration (p < 0.05), without changes in plasma creatinine, and therefore significantly increase glomerular filtration rate (GFR) measured by creatinine clearance (p < 0.05) in aged SHR. Urine flow, protein excretion and urine protein/creatinine ratio were also increased significantly with aging, and losartan decreased urine protein/creatinine ratio in aged SHR, but the difference was not significant.
in vascular smooth muscle cell growth, migration and ves-

el matrix alteration, and include mechanisms that increase 

production of vasoactive molecules such as Ang II and ET-1 

(Berk 2001). Results obtained from our study agree that Ang II 

plays an important role in hypertension and age dependent 

vascular remodeling via type-1 receptors, thus the blockade 

of Ang II AT1 receptors with losartan significantly improved 

haemodynamics in aorta of adult as well aged SHR, however, 

AVR was still higher in aged than in adult SHRs, while haemo-

dynamics in carotid artery was improved only in aged SHRs. 

Thus the beneficial effects of Ang II AT1 receptor antagonist 

on structure and function of the conductive arteries could 

account for the decline of CV risk in old rats. 

Age-induced increase in total cholesterol and triglyceride 

levels, were not changed after 4 weeks of antihypertensive 

treatment with losartan. These results are in agreement with 

results obtained from study of Andrzejczak et al. (2007) 

who found non-significant difference in total cholesterol 

and high density lipoprotein level after enalapril, quinapril and 

losartan treatment in SHR. 

We evaluated age-related kidney function through GFR 

and urinary protein/creatinine ratio as a marker of pro-

teinuria (Krishna et al. 1987). Our results show that aging 

markedly reduced GFR in hypertensive rats. Chronic ad-

ministration of Ang II receptor blocker, losartan, induced 

the significant increase in GFR in aged, without any effect 

in adult SHR. Tank et al. (1994) has obtained greater reduc-

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in adult SHR. Tank et al. (1994) has obtained greater reduc-

tion in GFR and renal plasma flow in 15-month-old than 

in 3-month-old SHRs. They also found that responses to 

systemic vasoconstrictor stimuli, such as Ang II and ET-1, 

were exaggerated in the aging kidney, whereas responsiveness 

to losartan was preserved but not enhanced. Urinary pro-

tein/creatinine ratio was markedly elevated in both untreated 

and losartan-treated aged SHRs, as a consequence of renal 

disease progression, and losartan slightly attenuate age and 

hypertension-induced proteinuria. Taking all together, we 

conclude that protective effects of AT1 receptor blockade 

on renal function could be in part due to dilatory effects of 

Ang II blocker on efferent and afferent arterioles and aug-

mentation of glomerular membrane permeability. 

In summary, these results demonstrated that regional 

haemodynamics and LVH, as well GFR were significantly 

impaired in SHRs with advanced age. Moreover, presented 
data indicates that losartan within 4 weeks, beside its ben-
ficial effects on haemodynamics, LVH and kidney function, 
also regresses RV weight, and therefore further decreases CV 

risk in aged hypertensive rats. This could contribute to better 
outcomes in elderly humans with hypertensive cardiac and 
renal complications. 

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